

Toxicity assessments of waters from the Grand Calumet River area of concern, 2018

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My background

I have over 20 years of experience as an environmental toxicologist developing and applying assessment tools using *Daphnia* and other species.

- Member of the US EPA FIFRA science advisory panel
- Contributed to the development of WET testing methods
- Helped validate rapid bioassessment protocol III
- Co-founded the *Daphnia* genomics consortium and developed many publicly available genomics resources for *D pulex*
- Worked to establish *D. pulex* as a NIH model species
- ...and according to teenage kids a real *Daphnia* geek

Rationale for our work

These tests are needed to meet the proposed criteria for removal from the AOC of:

Beneficial use impairment 13: Degradation of phytoplankton and zooplankton populations.

BUI #13 removal targets adopted by the state

This BUI can be considered for removal when:

- There are no violations of the minimum dissolved oxygen concentrations in the AOC;
- Levels of chlorophyll-a are consistent with IDEM “fully supporting” levels throughout the AOC; and
- Waters within the Grand Calumet River AOC are not listed as impaired due to degradation of phytoplankton or zooplankton in the most recent Indiana Integrated Water Monitoring and Assessment Report (submitted to U.S. EPA every two years) and/or the most recent Indiana Fish Consumption Advisory.

Proposed additional considerations for removal

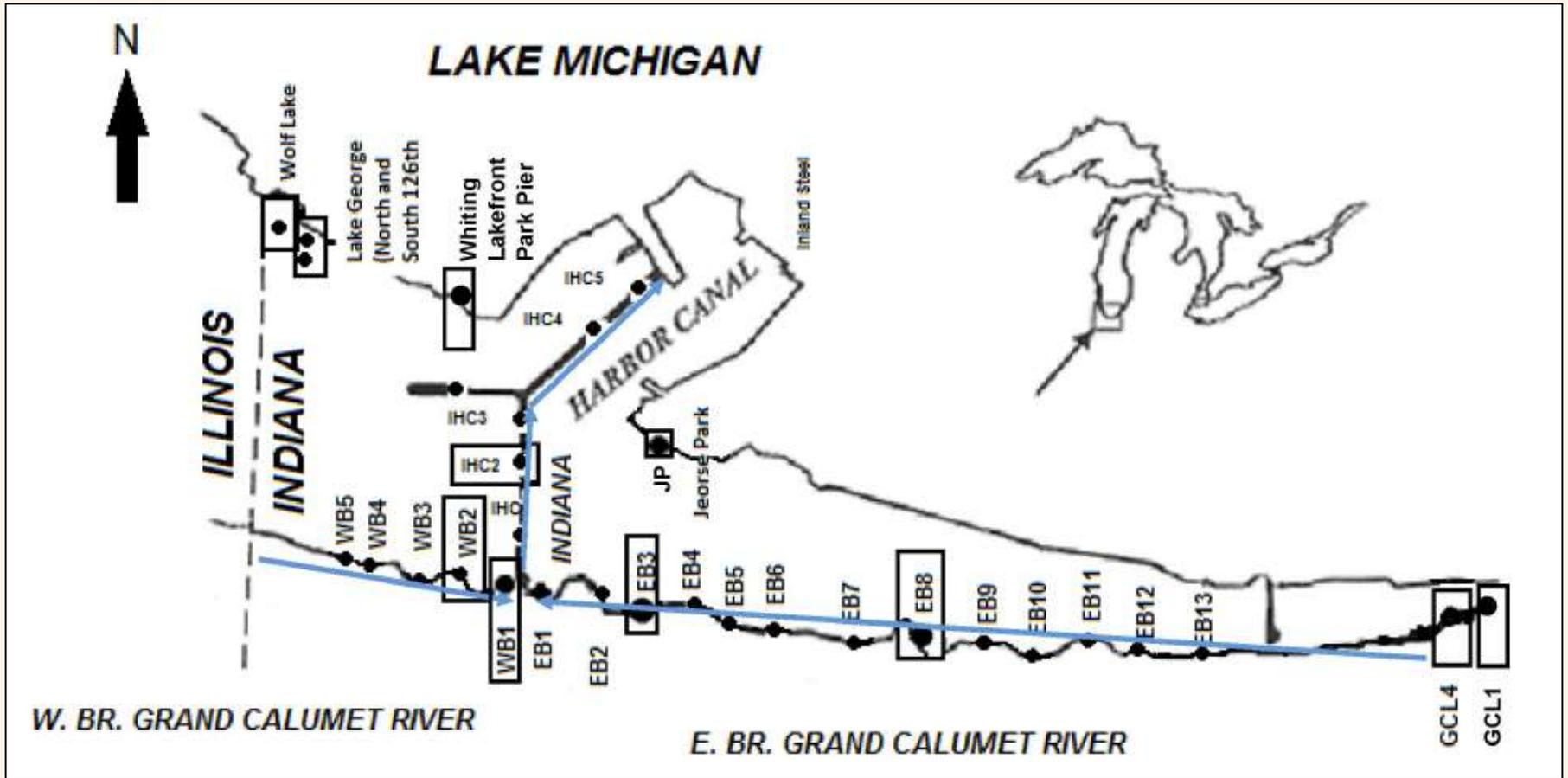
Simon, 2015

- Phytoplankton and zooplankton population targets are met for species richness, diversity, evenness, similarity indices consistent with Lake Michigan measures and expected seasonal differences.
- **No significant difference in mortality, mobility, or algal stimulation is demonstrated compared to an appropriate control or Lake Michigan.**
- Additional Lake Michigan species richness, and diversity indices measurements from Non-AOC sites (Mt. Baldy, Dune Acres, or other appropriate sites) are compared with those from the AOC. By virtue of being outside the GCR AOC, such non-AOC sites are presumed to reflect unimpaired ambient conditions.

Assessment criteria for additional considerations

Parameter	Mean Protection Values (Simon 2015)	Lake Michigan
Species Richness	Not significantly different* than Lake Michigan ambient control**	Seasonal difference dependent
Phytoplankton	Not significantly different* than Lake Michigan ambient control**	12 (11-13 species)
Zooplankton	Not significantly different* than Lake Michigan ambient control**	7 (6-8 species)
Shannon-Weiner Index (H')	Not significantly different* than Lake Michigan ambient control**	0.805-2.483
Evenness	Not significantly different* than Lake Michigan ambient control**	0.805-1.000
Jaccard Coefficient (S _J)	Not significantly different* than Lake Michigan ambient control**	38.89-43.75% similarity
<i>Selenastrum</i> bioassay	Not significantly different* from a laboratory or ambient control**	Not significantly different from Lake Michigan ambient Control
Inhibition/Growth	< +/- 200%	Not significantly different than laboratory control or Lake Michigan ambient control
<i>Daphnia</i> acute or chronic bioassay	Not significantly different from a laboratory or ambient control	Not significantly different from Lake Michigan ambient control
survivorship	Not significantly different than laboratory control or Lake Michigan ambient control	Not significantly different than laboratory control
Mobility	Not significantly different than laboratory control or Lake Michigan ambient control	Not significantly different than laboratory control

The study area

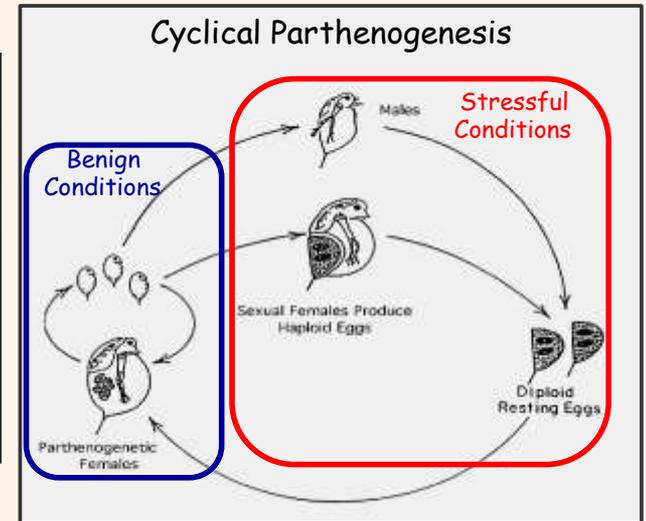
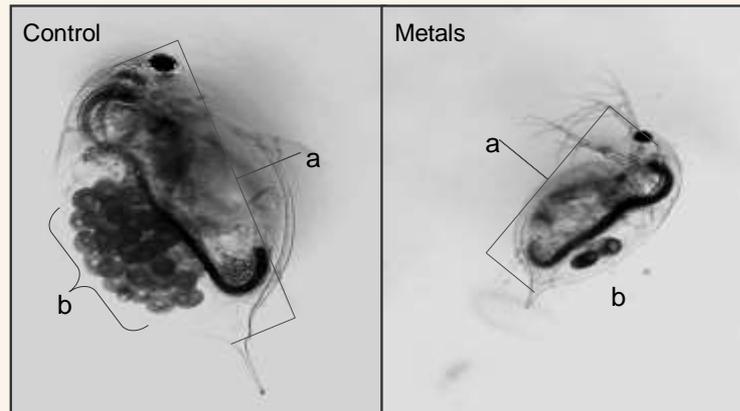


Map of the Grand Calumet River with sampling sites indicated by boxes.

The work plan

- Two sampling periods in 2018, June and August
- 12 sites were sampled each period
- Each site was assessed using:
 - Acute toxicity assays with the zooplankton, *Daphnia pulex* (EPA SOP#2024, Test method 2021.0)
 - Subchronic toxicity assays with *D. pulex* (EPA 2002, Test methods 2021.0)
 - Growth/inhibition assays with the phytoplankton, formerly known as *Selenastrum capricornutum* (EPA SOP #2027, Test method 1003.0)

Daphnia are environmental sentinels and lab models



***Daphnia* is the most commonly used species in aquatic toxicity testing!**

*"What's in a name? That which we call a rose
By any other name would smell as sweet."*



*Selenastrum capricornutum =
Pseudokirchneriella subcapitata =
Ankistrodesmus subcapitatus =
Kirchneriella subcapitata =
Raphidocelis subcapitata*

It is the most frequently used ecotoxicological bioindicator species of algae, because it has a high growth rate, is relatively sensitive to toxicants, and results are highly reproducible compared to other algae.

Acute *D. pulex* toxicity: June

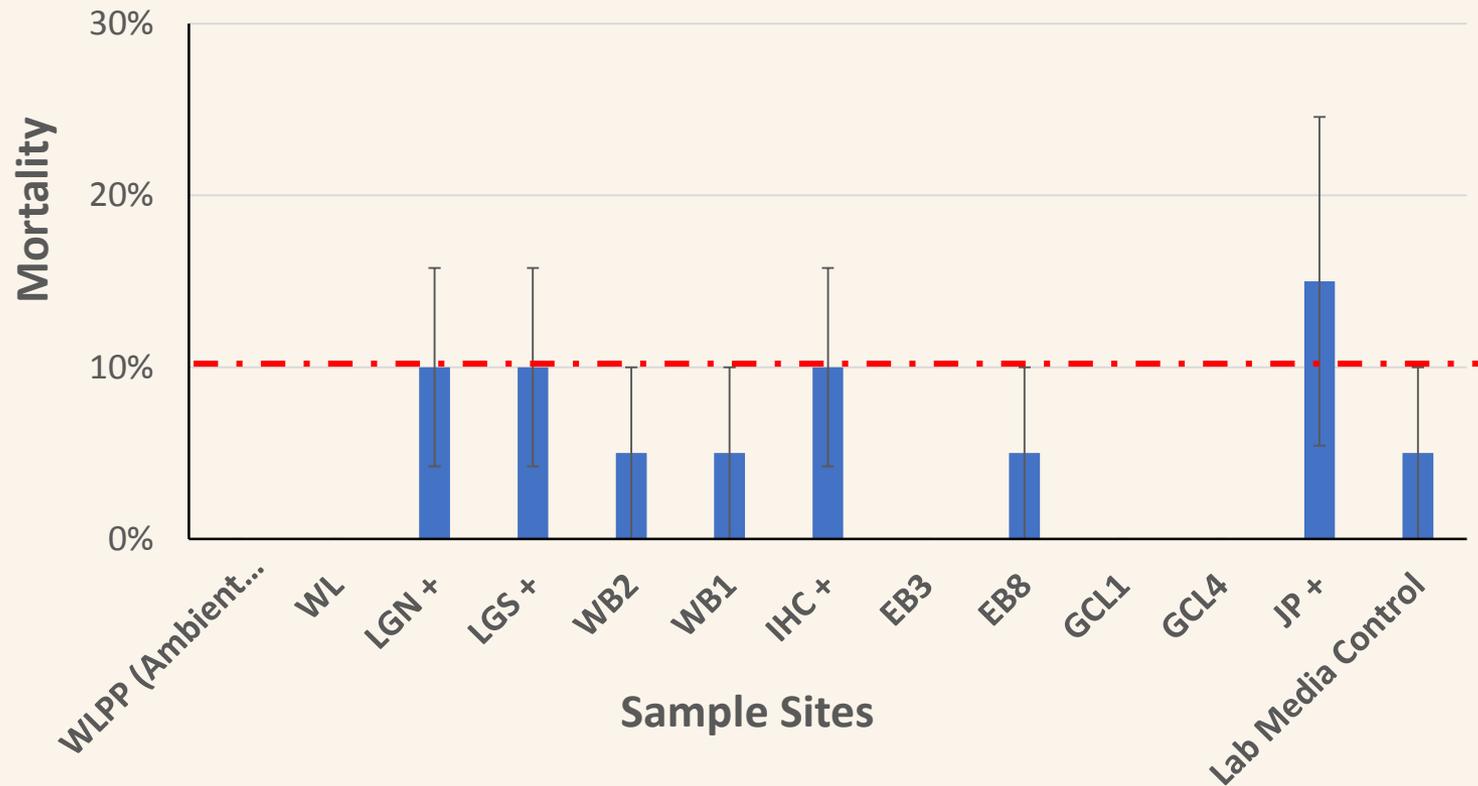
	Grand Calumet Sampling	June 2018	Acute Test
Site Code	Sample Site ID		Mortality
WB1	AB33379		5%
WB2	AB33380		5%
EB3	AB33381		0%
EB8	AB33382		5%
GCL1	AB33383		0%
GCL4	AB33384		0%
IHC	AB33385		10%#
JP	AB33386		15%#
LGN	AB33387		10%#
LGS	AB33388		10%#
WL	AB33389		0%
WLPP	AB33390 (Ambient Control)		0%
COMBO	Combo Control		5%
Notes:	# Sample used in Serial Dilution Acute Toxicity Test		

Acute *D. pulex* serial dilution toxicity tests

Site Code	Site ID	Sample Concentration	Mortality	Std Error
COMBO	Control	100%	5.0%	5.0%
IHC	AB33385	6.25%	5.3%	5.0%
IHC	AB33385	12.5%	5.0%	5.0%
IHC	AB33385	25%	15.0%	9.6%
IHC	AB33385	50%	10.0%	5.8%
IHC	AB33385	100%	10.0%	5.8%
JP	AB33386	6.25%	0.0%	0.0%
JP	AB33386	12.5%	10.0%	5.8%
JP	AB33386	25%	5.0%	5.0%
JP	AB33386	50%	0.0%	0.0%
JP	AB33386	100%	0.0%	0.0%
LGN	AB33387	6.25%	15.0%	5.0%
LGN	AB33387	12.5%	10.0%	10.0%
LGN	AB33387	25%	15.0%	5.0%
LGN	AB33387	50%	10.0%	5.8%
LGN	AB33387	100%	0.0%	0.0%
LGS	AB33388	6.25%	5.0%	5.0%
LGS	AB33388	12.5%	5.0%	5.0%
LGS	AB33388	25%	5.0%	5.0%
LGS	AB33388	50%	5.0%	5.0%
LGS	AB33388	100%	0.0%	0.0%

What does this mean? NOISE!

Zooplankton Acute Toxicity Test
Grand Calumet June 2018 Sampling



Acute *D. pulex* toxicity: August

	Grand Calumet Sampling	August 2018 Acute Test
Site Code	Sample Site ID	Mortality
WB1	AB33708	4%
WB2	AB33709	0%
EB3	AB33710	4%
EB8	AB33714	12%
GCL1	AB33715	0%
GCL4	AB33716	0%
IHC	AB33711	4%
JP	AB33717	0%
LGN	AB33718	4%
LGS	AB33719	4%
WL	AB33712	0%
WLPP	AB33713 (Ambient Control)	0%
COMBO	Combo Control	0%
Notes:	No Serial Dilution Acute Toxicity Tests warranted due to low mortality rates and results of June 2018 tests.	

Subchronic *D. pulex* toxicity: June

	Grand Calumet June 2018 Sampling	Subchronic Test	Subchronic Test	Subchronic Test
Site Code	Sample Site ID	Avg # Young/Adult	Combo Control Normalized (%)	Ambient Control Normalized (%)*
WB1	AB33379	14.1	20	0.0
WB2	AB33380	15.3	30	10
EB3	AB33381	13.6	20	0.0
EB8	AB33382	6.6	-40	-50
GCL1	AB33383	8.9	-20	-40
GCL4	AB33384	13.9	20	0.0
IHC	AB33385	11.8	0.0	-20
JP	AB33386	14.9	30	10
LGN	AB33387	9.2	-20	-30
LGS	AB33388	9.9	-20	-30
WL	AB33389	17.1	0.50	20
WLPP	AB33390 (Ambient Control)	9.6	-20	X
COMBO	Combo Control	10.0	x	-30

KEY
Sign Higher
Sign Lower
Sign @ p<0.1
No Sign Diff

Notes: *Ambient Control is L. Michigan Whiting Lakefront Park Pier sampling site

Subchronic *D. pulex* toxicity: August

	Grand Calumet August 2018 Sampling	Subchronic Test	Subchronic Test	Subchronic Test
Site Code	Sample Site ID	Avg # Young/Adult	Combo Control Normalized (%)	Ambient Control Normalized (%)*
WB1	AB33708	31.1	150	80
WB2	AB33709	19.3	60	10
EB3	AB33710	24.1	100	40
EB8	AB33714	29.6	140	70
GCL1	AB33715	11.3	-10	-40
GCL4	AB33716	13.9	10	-20
IHC	AB33711	20.7	70	20
JP	AB33717	17.6	40	0.0
LGN	AB33718	16.9	40	0.0
LGS	AB33719	17.9	50	0.0
WL	AB33712	19.1	60	10
WLPP	AB33713 (Ambient Control)	17.5	40	X
COMBO	Combo Control	12.2	X	-60

KEY
Sign Higher
Sign Lower
Sign @ p<0.1
No Sign Diff

Notes: *Ambient Control is L. Michigan Whiting Lakefront Park Pier sampling site

Phytoplankton growth/inhibition assay: June

Grand Calumet June 2018 Sampling		Phyto Test	Phyto Test
Site Code	Sample Site ID	Combo Control Normalized (%)	Ambient Control Normalized (%)*
WB1	AB33379	-11.7	13.3
WB2	AB33380	-4.5	22.6
EB3	AB33381	-25.7	-4.6
EB8	AB33382	-18.0	5.3
GCL1	AB33383	-13.0	11.7
GCL4	AB33384	-23.3	-1.5
IHC	AB33385	-23.6	-1.9
JP	AB33386	-32.3	-13.1
LGN	AB33387	-24.0	-2.4
LGS	AB33388	-62.9	-52.4
WL	AB33389	-54.3	-41.3
WLPP	AB33390 (Ambient Control)	-22.1	X
COMBO	Combo Control	X	28.4

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Notes: *Ambient Control is L. Michigan Whiting Lakefront Park Pier sampling site

Phytoplankton growth/inhibition assay: August

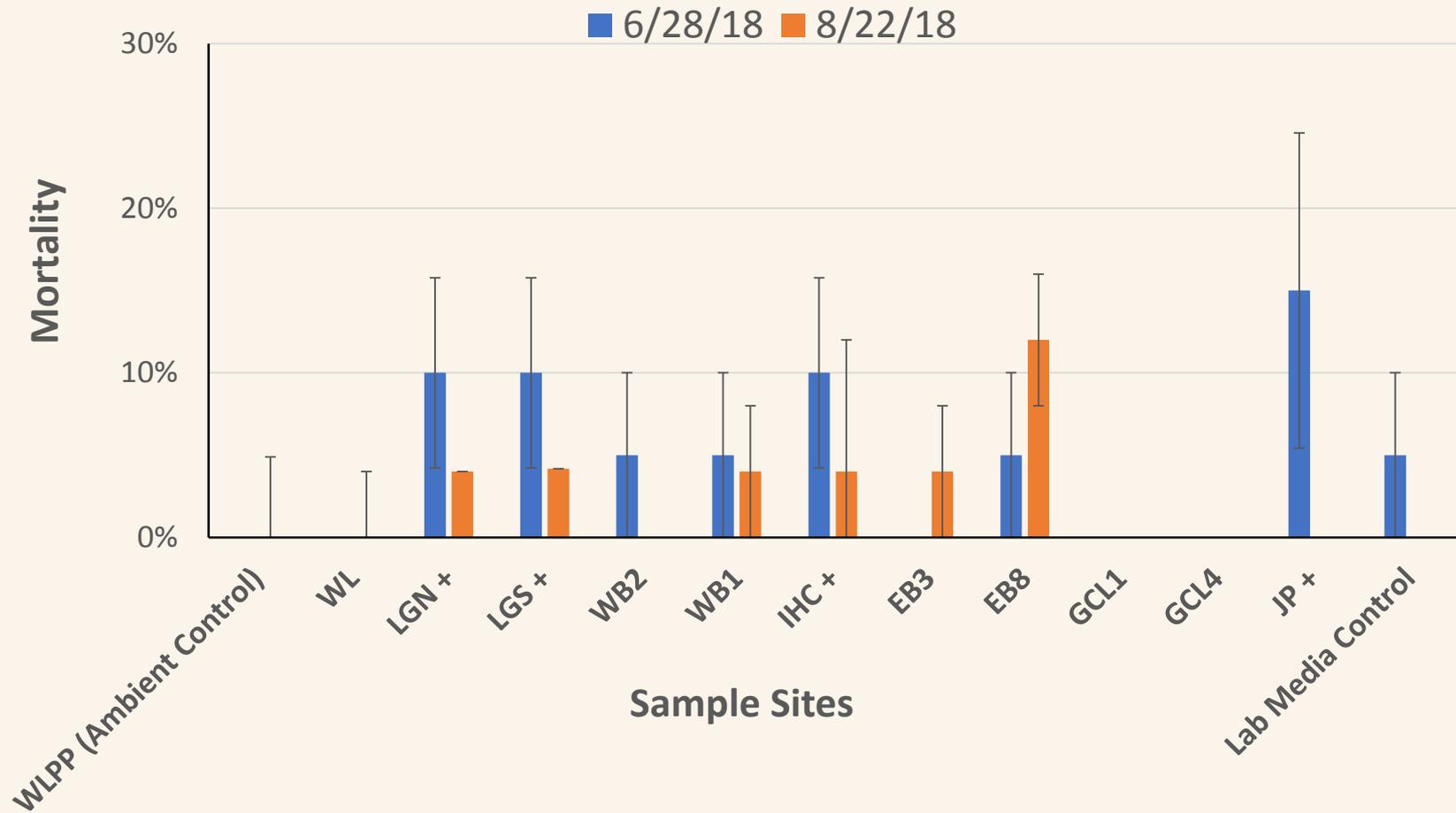
Grand Calumet June 2018 Sampling			
		Phyto Test	Phyto Test
Site Code	Sample Site ID	Combo Control Normalized (%)	Ambient Control Normalized (%)*
WB1	AB33379	36.5	43.9
WB2	AB33380	111.5	123.0
EB3	AB33381	71.8	81.1
EB8	AB33382	35.9	43.2
GCL1	AB33383	2.6	8.1
GCL4	AB33384	2.6	8.1
IHC	AB33385	40.4	48.0
JP	AB33386	-24.4	-20.3
LGN	AB33387	16.7	23.0
LGS	AB33388	-58.3	-56.1
WL	AB33389	-63.1	-61.1
WLPP	AB33390 (Ambient Control)	-5.1	X
COMBO	Combo Control	X	5.4

KEY
Sign Higher
Sign Lower
Sign @ p<0.1
No Sign Diff

Notes: *Ambient Control is L. Michigan Whiting Lakefront Park Pier sampling site

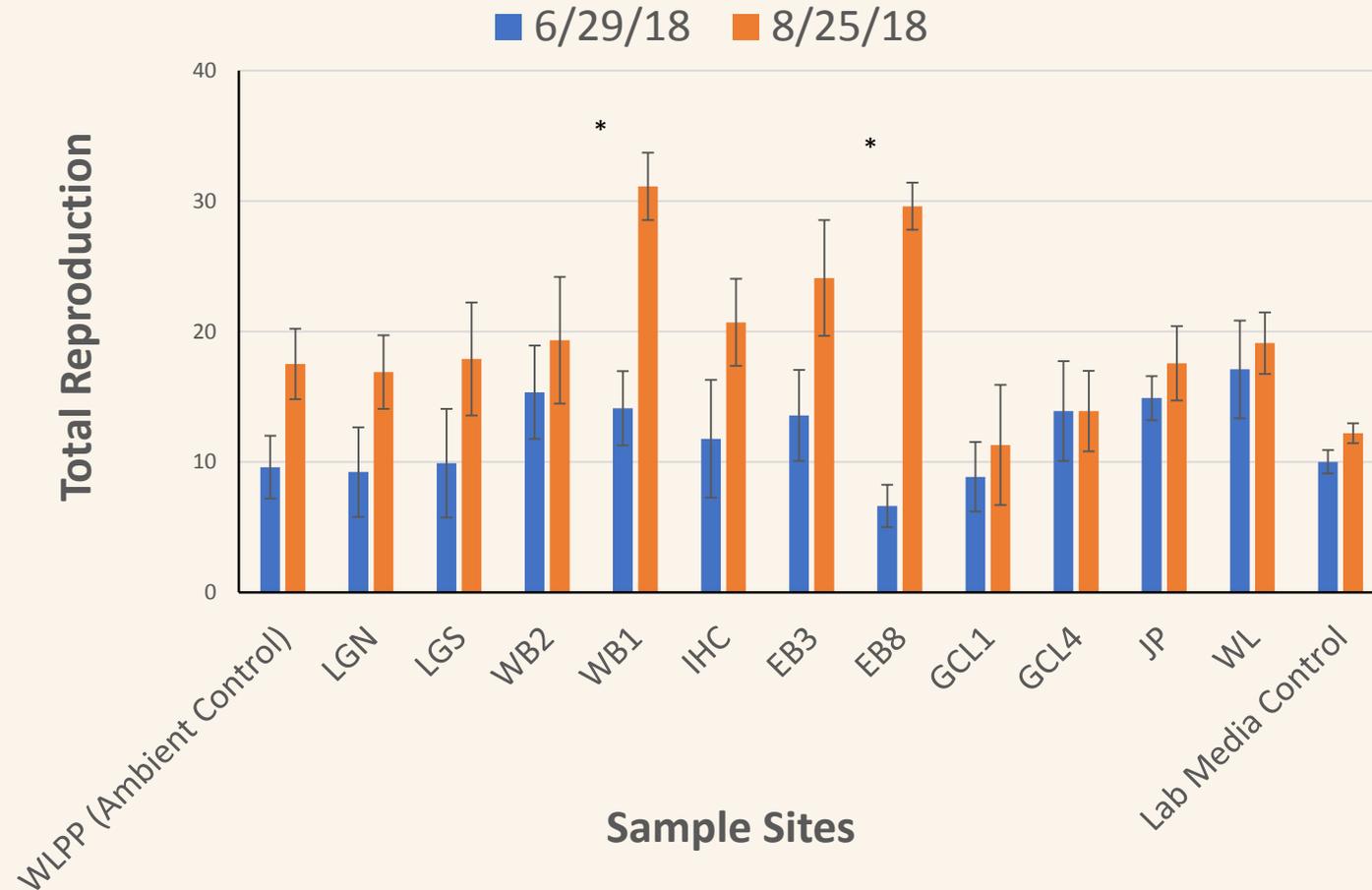
Acute *D. pulex* toxicity: Temporal comparisons

Zooplankton Acute Toxicity Test
Grand Calumet 2018 Sampling



Subchronic *D. pulex* toxicity: Temporal comparisons

Zooplankton Subchronic Toxicity Test
Grand Calumet 2018 Sampling



Phytoplankton growth/inhibition: Temporal comparisons

Phytoplankton Growth/Inhibition Test
Grand Calumet 2018 Sampling



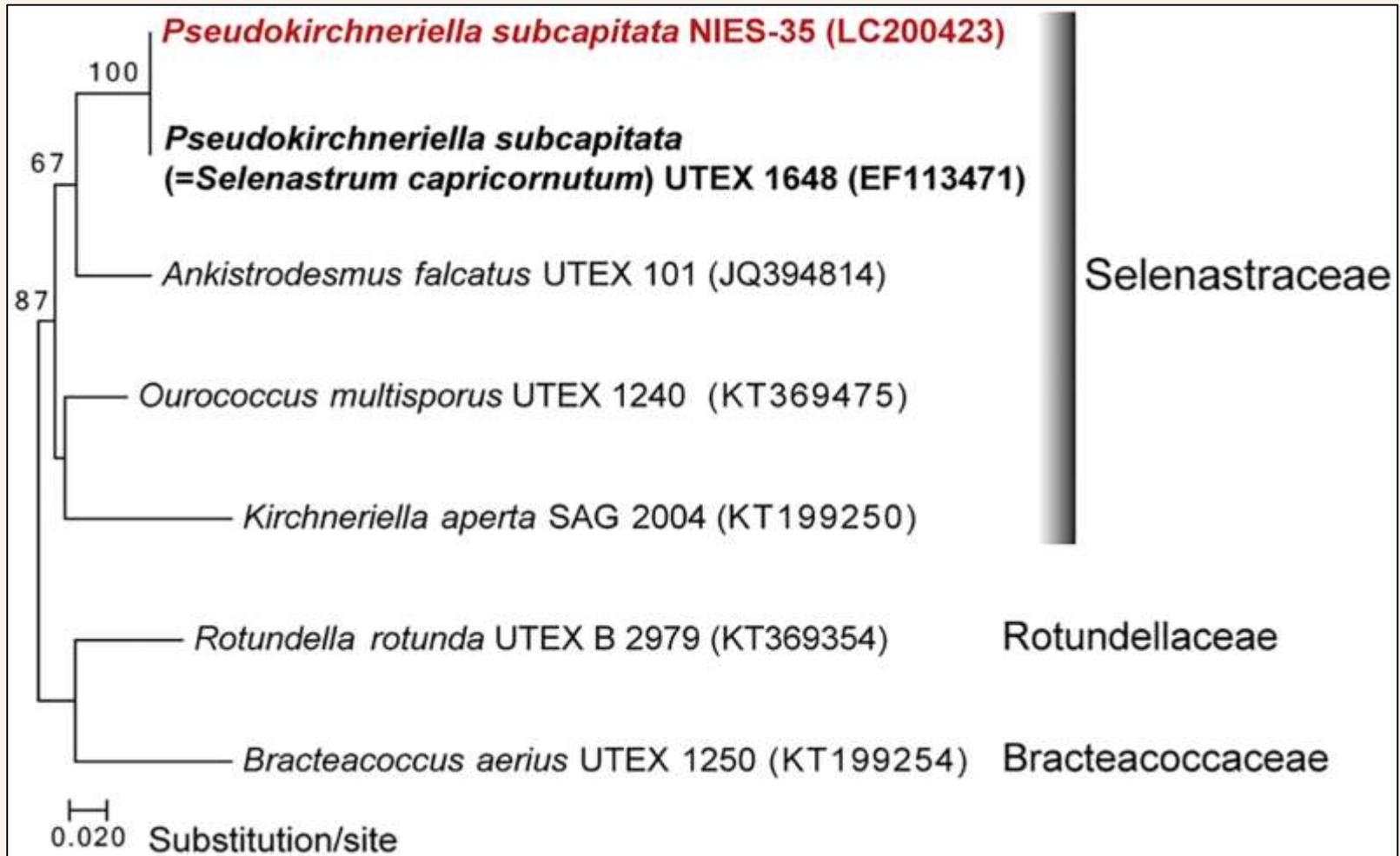
Comparison with Simon, 2015

- ***D. pulex* acute:** No acute toxicity was observed in the June or August tests, while ED8 and WB1 were highly toxic in Simon (2015), 50 and 40% respectively.
- ***D. pulex* subchronic:** All sites were tested during both sampling periods and significant stimulated reproduction was observed in WB1 (80%) and ED8 (70%) during the August tests. This was consistent with the stimulated reproduction observed in the 2015 tests at these same sites (WB1, 202%; ED8 254%), which were the only tested.
- **Phytoplankton growth/inhibition:** We observed consistent (i.e., both tests) and significant phytoplankton growth in WB1 and WB2 and inhibition in JP, LGN, and WL. With the exception of WB1 and JP, the 2015 tests revealed these same patterns. However, imposing the +/- 200% applied in the Simon report eliminates all significant results observed in the 2018 tests.

What does this mean for BUI13

- **Acute *D. pulex*** toxicity meets the proposed removal criteria for all waters tested. There was no toxicity.
- **Subchronic *D. pulex*** toxicity tests revealed significantly stimulated reproduction in WB1 and ED8. These sites also stimulated reproduction in tests reported in the 2015 report, adding support for these findings. However, the stimulated reproduction was not consistent across the two sampling periods in 2018.
- ***Phytoplankton growth and inhibition*** assays showed consistent significant differences compared to the Lake Michigan reference. However, none of these meets the +/-200% threshold, so meeting the removal target depends on the application of the threshold.

Phylogeny of *Selenastraceae*



Maximum likelihood tree based on the Rubisco large subunit.

Yamagishi et al., 2017, *PLoS One*, 12:e0171259